43. (New) A viral particle obtained from the retroviral vector delivery system of claim 1 for use in medicine.

44. (New) A cell transfected or transduced with the retroviral vector delivery system of claim 1 for use in medicine.

45. (New) A method of delivering a gene to a target cell comprising transfecting or transducing a target cell with a retroviral particle obtained from the retroviral vector delivery system of claim 1.

REMARKS

Applicants request reconsideration of the present application.

I. Status of the Claims

Following entry of this amendment, claims 1-26 and 28-45 are pending.

Claims 1-26, 28, 29, and 31-42 are amended without prejudice or disclaimer, solely to facilitate prosecution. In addition, claims 43-45 have been added to the application.

Claims 1-26, 29, 31-38, and 40-42 were amended to recite a retroviral "delivery system." Claim 1 was also amended to delete the terms "capable" and "derived from," and to more clearly and particularly state the invention. Claims 21, 22, and 41 were also amended to delete the phrase "derived from;" claims 1, 2, 13, 33, 36-38, and 40-42 were amended to delete the term "capable;" claims 9, 11, 24, 25, 29, 34, 40, and 41 were amended to delete the term "obtainable;" claim 12 was amended to delete the term "preventable;" and claims 26 and 28 were amended to more clearly state the invention.

New claims 43-45 recite subject matter cancelled from original claims 26 and 28.

Because the amendments to the claims do not introduce new matter, entry thereof by the Examiner is respectfully requested.

II. Summary of the Invention

The claimed invention is directed to a novel retroviral vector delivery system which efficiently expresses one or more nucleotides of interest (NOIs) at one or more target sites. *See* page 24, lines 24-28, of the application. In addition, the claimed delivery system provides high titres of vector virion which are safe for *in vivo* use. *See* page 24, lines 30-31, of the application.

Preferably, the delivery system of the invention comprises (1) a first nucleotide sequence ("NS") encoding a functional splice donor site, and (2) a second NS encoding a functional splice acceptor site. The functional splice donor site and the functional splice acceptor site flank a first nucleotide sequence of interest ("NOI"), the functional splice donor site is upstream of the functional splice acceptor site, and the retroviral vector is formed as a result of reverse transcription of a retroviral pro-vector. In addition, the retroviral pro-vector comprises (1) a first nucleotide sequence ("NS") encoding the splice donor site, and (2) a second NS encoding the splice acceptor site. The first NS is downstream of the second NS; such that the retroviral vector site is formed as a result of reverse transcription of the retroviral pro-vector. *See* page 25, lines 4-13, of the application.

In contrast to prior art retroviral delivery systems, the claimed invention provides stable gene expression, rather than transient gene expression. Specifically, the retroviral particles generated from primary target cells can transduce secondary target cells, and gene expression in the secondary target cells is stably maintained because of the integration of the retroviral genome into the host cell genome. The secondary target cells do not express significant amounts of viral protein antigens and, therefore, are less immunogenic than cells transduced with adenoviral vector. *See* page 45, lines 5-12, of the application. Such retroviral integration also enables the stable expression of therapeutic genes in target tissues. *See* page 45, lines 16-18, of the application.

Moreover, the use of a retroviral vector as the secondary vector enables targeting of specific cell types, such as rapidly dividing cells, as well as limited gene expression to a primary or secondary target site, thus eliminating the possible toxicity or antigenicty of an NOI. *See* page 45, lines 20-25, of the application.

As discussed in more detail below, the claimed invention is not taught or suggested by the cited prior art.

III. Rejections of the Claims Under 35 U.S.C. § 112, Second Paragraph

Claims 1-26, 28, 30, and 42 were rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to distinctly point out and claim the subject matter that Applicants regard as their invention. Office Action at page 2. Applicants respectfully traverse this ground for rejection.

Claim 1 was rejected as it is allegedly unclear "whether the retroviral provector described in the claim is or is not capable of producing a retroviral vector as described in the first phrase, because it is uncertain whether the retroviral vector has functional splice donor and acceptor sites . . ." Office Action at page 2.

Claim 1 has been amended to recite first and second nucleotide sequences "encoding" functional splice donor and acceptor sites, respectively. In addition, the claim has been amended to more clearly state the invention.

Claims 4, 7, 8, 11, and 12 were rejected as the "retroviral vector as defined is not consistent with the construction of the provector." Office Action at page 3. Claims 4, 7, 8, 11, and 12 have been amended to recite a retroviral vector "delivery system." In addition, in view of the amendments to claim 1, the definition of the claimed retroviral vector is now consistent with the construction of the provector.

Claims 1, 21, and 22 were rejected for recitation of the term "derived." While Applicants respectfully disagree with this ground for rejection, claims 1, 21, and 22, and claim 41, which also recited this term, have been amended to delete recitation of the term "derived" for the sole purpose of advancing the prosecution of this case.

Claims 1-3, 13, and 19 were rejected for recitation of the term "capable." Office Action at pages 3-4. While Applicants respectfully disagree with this ground for rejection, claims 1, 2, and 13, and claims 33, 36-38, and 40-42, which also recited this term, have been amended to delete recitation of the term "capable" for the sole purpose of advancing the prosecution of this case. (Claim 3 was not amended as is does not recite the term "capable.")

Claims 9, 11, 24, and 25 were rejected for recitation of the term "obtainable." Office Action at page 4. While Applicants respectfully disagree with this ground for rejection, claims 9, 11, 24, and 25, and claims 29, 34, 40, and 41, which also recited this term, have been amended to delete recitation of the term "obtainable" for the sole purpose of advancing the prosecution of this case.

Claim 12 was rejected for recitation of the term "preventable." Office Action at page 4. While Applicants respectfully disagree with this ground for rejection, claim 12 has been amended to delete recitation of the term "preventable" for the sole purpose of advancing the prosecution of this case.

Claim 25 was rejected as it was allegedly unclear whether the claim was directed to a retroviral particle or a cell. Office Action at page 4. While Applicants respectfully disagree with this ground for rejection, claim 25 has been amended to clarify that the claim is directed to a cell.

Claim 26 was rejected as it was allegedly unclear whether the claim was directed to a retroviral particle, a viral particle, a cell, or a retroviral particle. Office Action at pages 4-5. While Applicants respectfully disagree with this ground for rejection, claim 26 has been amended to clarify that the claim is directed to a retroviral delivery system, and claims 43 and 44, which recite the subject matter cancelled from claim 26, have been added to the application.

Finally, claim 28 was rejected as it was allegedly unclear whether the claim is directed to a method or a viral particle. Office Action at page 5. While Applicants respectfully disagree with this ground for rejection, claim 28 has been amended to clarify that the claim is directed to a method, and claim 45, which recites subject matter cancelled from claim 28, has been added to the application.

Because Applicants' claims are definite, withdrawal of this ground for rejection is respectfully requested.

IV. Rejection of the Claims Under 35 U.S.C. § 112, First Paragraph

Claims 1-26, 28, 30, and 42 were rejected under 35 U.S.C. § 112, first paragraph, as the specification allegedly does not enable a person of ordinary skill to use to make and use the invention. Office Action at pages 5-11. Applicants respectfully traverse this ground for rejection.

A. The Presence of Cryptic Splice Sites Does Not Render the Claimed Invention Non-Enabled

The claimed invention relates to novel retroviral vector delivery systems, comprising functional splice donor and acceptor sites, and which enable efficient intron inclusion.

In one embodiment, a synthetic splice donor (SD), derived from the simian virus 40 (SV40) small-T antigen gene (st-SD), is inserted between U3 and R of the 3' LTR (*see* page 68, lines 5-10, of the application), and a synthetic consensus splice acceptor (c-SA) is incorporated downstream of the packaging signal (*see* page 68, lines 12-17, of the application). A viral wild-type SD (wt-SD) and a cryptic SD (cr-SD) are disabled by GT-GC alteration (in the DNA sequence) or a GU-GC alteration in the GC sequence (*see* page 68, lines 19-24, of the application).

The st-SD is a strong splice donor. For example, comparative studies have demonstrated that the st-SD is actually more effective than a consensus sequence. *See* Ismail et al., *J. Virol.*, *74*(5):2367, col. 1, para. 2 (2000), cited by the Examiner. Likewise, the c-SA is a strong splice acceptor as it is a consensus sequence. Thus, for this embodiment of the invention, on reverse transcription the resulting LTR-expressed transcripts contain, in the 5' termini, the "very efficient" st-SD capable of interacting with the consensus SA downstream.

1. The Specification Enables The Use of all Potential NOIs in the Claimed Invention, Without Undue Experimentation

The Examiner alleged that the possible presence of cryptic splice sites in an NOI would hinder application of the method of the claimed invention. Applicants respectfully disagree with the Examiner's analysis and conclusion.

Because the potential presence of cryptic splice sites in retroviral transcripts has been well established, such cryptic splice sites would not hinder application of the claimed invention, as one of ordinary skill in the art at the time the claimed invention was made would be aware of their existence. Moreover, such a person of skill in the art would be able to compensate for such cryptic sites by, for example, identifying and altering the sites. This process was adopted, for example, for CAT gene constructs. *See* Ismail et al. (2000).

Typically, if a SA site is found to exist in an NOI, it can be identified by sequence analysis and removed. Even in the event that a cryptic splice site was not found to exist or was not removed, an analysis of any transcripts made would reveal the location of the splice site.

Moreover, it is highly unlikely that the skilled person would have to compensate for cryptic splice sites, because most NOI's are derived from cDNA and, therefore, would contain no or few active splice sites. In addition, it is highly unlikely that a *strong* SA would exist in a normal cellular transcript following conversion to cDNA.

Thus, even if a NOI should be tested for the presence of a cryptic splice site, the form of testing required by a skilled person would amount to routine experimentation.

B. The Use of Applicants' Claimed Retroviral Vector Delivery System in Gene Therapy is Enabled

Claims 20, 25, 26, and 28 were rejected as encompassing the use of retroviral vector delivery systems in gene therapy, which, according to the Examiner, is "incompletely proven and unpredictable." Office Action at page 9. Applicants respectfully disagree with the Examiner" analysis and conclusion.

Several of the vectors encompassed by the claimed invention are based on an MLV backbone. It has been well established that MLV vectors are capable of gene delivery *in vivo*. *See e.g.*, Jolly et al., *Cancer Gene Therapy, 1*:51-64 (1994), at page 51, col. 2, para. 2, which states that:

> *"These vectors, in the form of murine leukemia virus (MLV) and its relatives, have one of the longest pedigrees in gene transfer therapy. The first vector was described in 1981 and the first patient was treated (for adenosine deaminase deficiency) in 1990."*

Other vectors encompassed by the claimed invention are based on lentiviral vectors. It is also known that lentiviral vectors are suitable for gene therapy. For example, Verma et al., (1997), cited by the Examiner, states at pages 240-241 that:

> *"when lentivirus vectors are injected into rodent brain, liver, muscle, eye or pancreatic islet cells, they give sustained expression for over six months- the longest time so far."*

Accordingly, the delivery/transduction properties of the "split-intron vectors" of the present invention are well established in the prior art. Moreover, there is no reasonable expectation that the rules for splicing are different in cultured cells and in cells *in vivo*. Consequently, claims 20, 25, 26, and 28 are enabled for at least gene delivery/transfer to a target site.

V. Rejection of the Claims Under 35 U.S.C. § 102(b)

Claims 1-6, 9, 10, 12-14, and 18-25 were rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Morgenstern et al., *Nucleic Acids Research, 18*:3587 (1990). Office Action at page 11. Applicants respectfully traverse this ground for rejection.

Claims 15-17 were rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Takeda et al., *Nature, 314*:452-454 (1985). Office Action at page 15. Applicants respectfully traverse this ground for rejection.

Finally, claims 9-11 were rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Kriegler et al., *Cell, 38*:483-491 (1984). Office Action at page 15. Applicants respectfully traverse this ground for rejection.

A. The Claimed Invention is Patentable over Morgenstern et al.

Morgenstern et al. do not teach or suggest the claimed invention, as this reference merely teaches splice site inactivation by point mutation. Moreover, this reference is acknowledged by Applicants at page 17, lines 7-13; page 39, line 29; page 72, line 18; and Figure 27b, as being part of the prior art, and distinguishable from the claimed invention.

Finally, given the amendments to the claims, detailed above, it is courteously submitted that the claimed invention is patentable over Morgenstern et al., and withdrawal of this ground for rejection is respectfully requested.

B. The Claimed Invention is Patentable over Takeda et al.

The claimed invention is also patentable over Takeda et al. (1985). Although Takeda et al. (1985) disclose a recombinant retroviral vector carrying nucleotide sequences encoding immunological molecules, Takeda et al. (1985) do not disclose the novel retroviral vector of the claimed invention. Withdrawal of this ground for rejection is respectfully requested.

C. The Claimed Invention is Patentable over Kriegler et al.

Finally, the claimed invention is patentable over Kriegler et al. (1984). Although Kriegler et al. (1984) describe retroviral vectors containing the coding sequence for the early genes of SV40, they do not teach or suggest the novel retroviral vector of the claimed invention. Withdrawal of this ground for rejection is respectfully requested.

VI. Conclusion

In view of the foregoing amendments and remarks, Applicants respectfully submit that all of the pending claims are now in condition for allowance. An early notice to this effect is earnestly solicited.

Should the Examiner have any questions or comments regarding the pending application or this Amendment, the Examiner is requested to call the undersigned at 202-672-5538.

If there are any fees due in connection with the filing of this Amendment, please charge the fees to our Deposit Account No. 19-0741. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Date June 25, 2001 By Michele M. Simkin

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Versions with Markings to Show Changes Made

1. (Amended) A retroviral vector **delivery system** comprising:

(a) a ~~**first nucleotide sequence ("NS") encoding a**~~ functional splice donor site; and

(b) a ~~**second NS encoding a**~~ functional splice acceptor site;

wherein:

(i) the functional splice donor site and the functional splice acceptor site flank a first nucleotide sequence of interest ("NOI");

(ii) **[wherein]** the functional splice donor site is upstream of the functional splice acceptor site; **and**

(iii) **[wherein]** the retroviral vector is **[derived from]** **formed as a result of reverse transcription of** a retroviral pro-vector, wherein the retroviral pro-vector comprises:

(a) a first nucleotide sequence ("NS") **[capable of yielding the functional]** **encoding the** splice donor site; and

(b) a second NS **[capable of yielding the functional]** **encoding the** splice acceptor site;

wherein the first NS is downstream of the second NS; such that the retroviral vector **comprising a first NS encoding a functional splice donor site and a second NS encoding a functional splice acceptor site** is formed as a result of reverse transcription of the retroviral pro-vector.

2. (Amended) A retroviral vector **delivery system** according to claim 1 wherein the retroviral pro-vector comprises a third NS that is upstream of the second **[nucleotide sequence]** NS; wherein the third NS **[is capable of yielding]** **encodes** a non-functional splice donor site in **the retroviral vector**.

3. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the retroviral vector further comprises a second NOI; wherein the second NOI is downstream of the functional splice acceptor site.

4. (Twice Amended) A retroviral vector **delivery system** according to claim 3 wherein the retroviral pro-vector comprises the second NOI; wherein the second NOI is upstream of the second **[nucleotide sequence] NS**.

5. (Twice Amended) A retroviral vector **delivery system** according to claim 3 wherein the second NOI, or the expression product thereof, is or comprises a therapeutic agent or a diagnostic agent.

6. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the first NOI, or the expression product thereof, is or comprises any one or more of an agent conferring selectability, a viral essential element, or a part thereof, or combinations thereof.

7. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the first NS is at or near to the 3' end of a retroviral pro-vector.

8. (Twice Amended) A retroviral vector **delivery system** according to claim 7 wherein the first NS of the retroviral pro-vector comprises a third NOI; wherein the third NOI is any one or more of a transcriptional control element, a coding sequence, or a part thereof.

9. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the first NS **[is obtainable from a virus] is a viral NS**.

10. (Amended) A retroviral vector **delivery system** according to claim 9 wherein the first NS is an intron or a part thereof.

11. (Amended) A retroviral vector **delivery system** according to claim 10 wherein the intron is **[obtainable from]** the small t-intron of SV40 virus.

12. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the retroviral pro-vector comprises a retroviral packaging signal; and wherein the second NS is located downstream of the retroviral packaging signal such that splicing is **[preventable] prevented** at a primary target site.

13. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the second NS is placed downstream of the first NOI such that the first NOI is **[capable of being]** expressed at a primary target site.

14. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the second NS is placed upstream of a multiple cloning site such that one or more additional NOIs may be inserted.

15. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the second NS is a nucleotide sequence coding for an immunological molecule or a part thereof.

16. (Amended) A retroviral vector **delivery system** according to claim 15 wherein the immunological molecule is an immunoglobulin.

17. (Amended) A retroviral vector **delivery system** according to claim 16 wherein the second NS is a nucleotide sequence coding for an immunoglobulin heavy chain variable region.

18. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the vector additionally comprises a functional intron.

19. (Amended) A retroviral vector **delivery system** according to claim 18 wherein the functional intron is positioned so that it **[is capable of restricting] restricts** expression of at least one of the NOIs in a desired target site.

20. (Amended) A retroviral vector **delivery system** according to claim 19 wherein the target site is a cell.

21. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the vector or pro-vector is **[derivable from]** a murine oncoretrovirus or a lentivirus **retroviral vector or pro-vector**.

22. (Amended) A retroviral vector **delivery system** according to claim 21 wherein the vector is **[derivable from]** a MMLV, MSV, MMTV, HIV-1, or EIAV **retroviral vector**.

23. (Twice Amended) A retroviral vector **delivery system** as defined in claim 1 wherein the retroviral vector is an integrated provirus.

24. (Twice Amended) A retroviral particle **[obtainable]** obtained from a retroviral vector **delivery system** according to claim 1.

25. (Twice Amended) A cell transfected or transduced with a retroviral vector **delivery system** according to claim 1 or a **cell transfected or transduced with a** retroviral particle **[obtainable] obtained** from a retroviral vector according to claim 1.

26. (Twice Amended) A retroviral vector **delivery system** according to claim 1 **[or a viral particle obtainable from said retroviral vector or a cell transfected or transduced with said retroviral vector or said retroviral particle]** for use in medicine.

28. (Twice Amended) A method **[comprising] of delivering a gene to a target cell comprising** transfecting or transducing a **target** cell with a retroviral vector according to claim 1 **[or a viral particle from said retroviral vector].**

29. (Twice Amended) A **retroviral vector** delivery system **[for a retroviral vector]** according to claim 1 or a viral particle **[obtainable] obtained** from said retroviral vector or a cell transfected or transduced with said retroviral vector or said retroviral particle, wherein the delivery system comprises one or more non-retroviral expression vector(s), adenovirus(es), or plasmid(s) or combinations thereof for delivery of an NOI or a plurality of NOIs to a first target cell and a retroviral vector for delivery of an NOI or a plurality of NOIs to a second target cell.

31. (Twice Amended) A retroviral vector **delivery system** according to claim 1 comprising a functional intron that can restrict expression of one or more NOIs within a desired target cell.

32. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein the first NS is delivered by a reverse transcriptase from the 3' end of the retroviral pro-vector to the 5' end of the retroviral vector.

33. (Twice Amended) A hybrid viral vector **delivery** system for *in vivo* gene delivery**[, wherein the system comprises**] **comprising** one or more primary viral vectors which encode a secondary viral vector, wherein the primary vector or vectors **[is capable of infecting] infects** a first target cell and of expressing therein the secondary viral vector, **and** wherein the secondary vector **[is capable of transducing] transduces** a secondary target cell.

34. (Twice Amended) A hybrid viral vector **delivery** system according to claim 33 wherein the primary vector is **[obtainable] obtained** from or is based on a adenoviral vector and the secondary viral vector is **[obtainable] obtained** from or is based on a retroviral vector.

35. (Twice Amended) A hybrid viral vector **delivery** system according to claim 33 wherein the secondary viral vector is a lentiviral vector and said lentiviral vector has a split-intron configuration.

36. (Twice Amended) A hybrid viral vector **delivery** system according to claim 33 wherein the secondary viral vector is a lentiviral vector and the lentiviral vector comprises or **[is capable of delivering] delivers** a split-intron configuration.

37. (Amended) A lentiviral vector **delivery** system wherein the lentiviral vector comprises or **[is capable of delivering] delivers** a split-intron configuration.

38. (Amended) An adenoviral vector **delivery** system wherein the adenoviral vector comprises or **[is capable of delivering] delivers** a split-intron configuration.

39. (Amended) Vectors or plasmids based on or obtained from any one or more of the entities **selected from the group consisting of [presented as]** pE1splA, pCI-Neo, pE1RevE, pE1HORSE3.1, pE1PEGASUS4, pCI-Rab, **and** pE1Rab.

40. (Twice Amended) A hybrid viral vector **delivery** system for *in vivo* gene delivery, said system comprising a primary viral vector which encodes a secondary viral vector, wherein:

(a) the primary vector **[is capable of infecting] infects** a first target cell and **[of expressing] expresses** therein the secondary viral vector,

(b) **[wherein]** the secondary vector **[is capable of transducing] transduces** a secondary target cell, **and**

(c) **[wherein]** the primary vector is **[obtainable] obtained** from or is based on a adenoviral vector and the secondary viral vector is **[obtainable] obtained** from or is based on a retroviral vector.

41. (Twice Amended) A hybrid viral vector **delivery** system for *in vivo* gene delivery, said system comprising a primary viral vector which encodes a secondary viral vector, wherein:

(a) the primary vector **[is capable of infecting] infects** a first target cell and **[of expressing] expresses** therein the secondary viral vector,

(b) **[wherein]** the secondary vector **[is capable of transducing] transduces** a secondary target cell,

(c) **[wherein]** the primary vector is **[obtainable] obtained** from or is based on a adenoviral vector and the secondary viral vector is **[obtainable] obtained** from or is based on a retroviral vector; **and**

(d) **[wherein]** the viral vector system comprises:

(1) a functional splice donor site; and

(2) a functional splice acceptor site;

wherein:

(i) the functional splice donor site and the functional splice acceptor site flank a first nucleotide sequence of interest ("NOI");

(ii) **[wherein]** the functional splice donor site is upstream of the functional splice acceptor site;

(iii) **[wherein]** the retroviral vector is **[derived from]** **formed as a result of reverse transcription of** a retroviral pro-vector;

(iv) **[wherein]** the retroviral pro-vector comprises a first nucleotide sequence ("NS") **[capable of yielding]** **yields** the functional splice donor site and a second NS **[capable of yielding]** **yields** the functional splice acceptor site; **and**

(v) [wherein] the first NS is downstream of the second NS; such that the retroviral vector is formed as a result of reverse transcription of the retroviral pro-vector.

42. (Twice Amended) A retroviral vector **delivery system** according to claim 1 wherein said retroviral vector delivery system **[is capable of differential expression of]** **differentially expresses** NOIs in target cells.